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Gender-specific responses to winter defoliation of greenhouse-grown *Atriplex canescens* clones under contrasting soil moisture regimes

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Abstract

A greenhouse experiment with female, male and monecious clones (36 of each, 108 total) of tetraploid *Atriplex canescens* (Pursh) Nutt. (fourwing saltbush) was conducted to determine gender-specific responses to simulated herbivory under contrasting conditions of soil moisture. We imposed three levels of winter defoliation (control, 40%, and 70% stem removal) and two soil moisture treatments (control and water stress). Gender-related responses to defoliation were context-specific. Defoliated female clones produced significantly less shoot biomass than male or monecious clones when soil moisture conditions were not limiting. All genders produced similar amounts of shoot biomass under conditions of soil moisture stress. Root biomass responses exhibited the opposite trend. With adequate soil moisture all defoliated clones produced similar amounts of root biomass, but water-stressed defoliated females usually produced less root biomass than their male and monecious counterparts. Leaf gas-exchange measurements indicated that females were less efficient than males in the use of water. Monecious clones outperformed both females and males when conditions of stress were greatest. Defoliated female shrubs may be at a competitive disadvantage regardless of the

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environmental context, and could therefore be expected to be the shrub gender most negatively affected by herbivory.

Keywords: Fourwing saltbush; Photosynthesis; Herbivory; Environmental stress

1. Introduction

Gender-specific responses to physiological stress occur in many dioecious plant species (Freeman and McArthur, 1982; Zimmerman and Lechowicz, 1982; Crawford and Balfour, 1983; Krischik and Denno, 1990; Dawson and Ehleringer, 1993; Houssard et al. 1994). Because females are committed to larger reproductive expenditure than males, they are generally less able to survive under conditions of reduced resources and are therefore less abundant in less favourable microhabitats of the landscape. Gender-specific responses to defoliation, however, are not as consistent across plant species. Greater male susceptibility (Delph et al., 1993), no gender-specific differences (Krischik and Denno, 1990), or greater female susceptibility to defoliation (Oyama and Mendoza, 1990) have been found in different dioecious species. Since males are frequently less well defended than females, they often exhibit higher growth rates (Jing and Coley, 1990) and are therefore assumed to be better able to recover from herbivory than are females. However, if gender-specific responses are influenced by environmental conditions, as suggested by Boecklen and Hoffman (1993), differences in the rates at which females and males recover from herbivory may depend on the availability of critical resources for plant growth.

Female fourwing saltbush shrubs are less drought resistant than male shrubs (Freeman and McArthur, 1982) and therefore tend to be over represented in moister patches of the landscape (Freeman et al., 1993). It is not known, however, whether male and female shrubs respond differently to browsing, and whether gender-specific responses depend on soil moisture status. Slower regrowth rates, probably driven by lower concentrations of critical metabolites, could put browsed female shrubs at a competitive disadvantage, particularly in situations of female-biased herbivory (Tiedemann et al., 1987; Cibils et al., 2003). However, in situations of drought, moderate defoliation could attenuate such disadvantages by reducing transpiration surface and decreasing internal water stress (Brown, 1995 and references therein).

Fourwing saltbush stands at our research site are tetraploid and, therefore, have an additional gender group composed of monecious shrubs, all of which carry labile sex genotypes, that can shift sex expression from year to year in response to physiological stress (McArthur, 1977; McArthur and Freeman, 1982; McArthur et al., 1992). The competitive advantage conferred by sex phenotype lability should allow this gender group to out-perform both male and female shrubs, particularly under conditions of stress.

We hypothesized that shrub gender-specific responses to defoliation would be context-specific. We predicted that female shrubs would recover more slowly from simulated herbivory than male or monecious shrubs under conditions of adequate soil moisture, and that gender-specific responses to defoliation would disappear under conditions of water stress. We also predicted that monecious shrubs would out-perform both male and female shrubs. We tested these predictions in a greenhouse experiment with fourwing saltbush clones. The overall objective of our study was to investigate shrub gender-specific responses to defoliation under contrasting conditions of soil moisture.

2. Materials and methods

Plant material was collected from several sites within the USDA-ARS Central Plains Experimental Range, approximately 60 km north-east of Fort Collins, Colorado (40° 49′N 107° 47′W) during the fall of 1996. We harvested stem cuttings from 30 fourwing saltbush shrubs (ten female, ten male, and ten monecious) at five fairly distant sites. At each site we randomly selected six donor shrubs (two of each sexual phenotype) at least 20 m apart from each other, and clipped a total of 36 ten-centimeter-long cuttings from each donor shrub. We used collection, handling, and rooting protocols proposed by Wiesner and Johnson (1977) and McArthur et al. (1984, pp. 261–264).

Immediately after clipping, stems were put into a cooler in marked containers with a 20–20–20 N–P–K solution (8 g of fertilizer/liter water). All material was kept in this solution and refrigerated at 3–5 °C for no more than 48 h. Clippings were then dipped into rooting hormone powder (a commercial product containing indole-3-butyric acid), and planted in labelled trays with a substrate composed of a mixture of 1 part of peat-moss and three parts of sand. All trays were placed on a mist bench for approximately 45 days, where they were exposed to 4 to 12 s-long spray events at 8–24-min intervals. Clippings that produced roots were transplanted into standard commercial potting mixture in individually labelled Styrofoam cups and taken to a greenhouse environment where they were kept at a temperature of 18–20 °C. In January 1997, surviving clones were transplanted into labelled 2 l metal containers filled with sifted prairie sandy loam topsoil. Clones were nursed in the greenhouse environment through the growing season of 1997 to allow them to flower so as to determine whether surviving female and male clones exhibited sex phenotype lability (McArthur, 1977). However, clones did not flower either in the summer of 1997 or during our experiment in the following growing season; therefore, clone gender in this paper must be interpreted as meaning 'clones taken from a donor shrub exhibiting that gender'. In November 1997, surviving clones (approximately 30% of all clippings collected in the field) were transplanted into labelled 16 l plastic pots with sifted loamy prairie topsoil, where they remained for the rest of the experiment.

One hundred and eight clones taken from 18 parent fourwing saltbush shrubs (six females, six males, and six monecious) were used in a 9-month experiment that began in December 1997 and was designed to measure effects of defoliation and water stress on plant productivity-related variables. Air temperature in the greenhouse was monitored by placing temperature data loggers at either end of the experiment area approximately 1 m above the bench surface under shelters to protect sensors form direct sunlight radiation. We found no detectable temperature-related gradients, and observed no apparent differences in light intensity; therefore all pots were randomly assigned a position on the bench that remained unchanged throughout the experiment.

We applied clipping treatments in winter to simulate the most common pattern of shrub use at our site on the shortgrass steppe, where fourwing saltbush stands are mostly used as a winter browse resource for cattle (Shoop et al., 1985). Defoliation treatments consisted of one clipping event at the beginning of the experiment that removed either 40% (moderate) or 70% (heavy) of the length of all stems 5 cm or longer. The biomass removed from each clone was put into separate labelled bags, oven-dried, and weighed.

Soil moisture treatments were imposed by varying watering intervals. We installed gypsum resistance blocks in 12 pots (six controls and six water stressed) containing non-defoliated clones (four females, four males, and four monecious) and took two weekly ohmmeter readings to determine soil water status. Ohmmeter readings were transformed to soil water potential values (SWP) using previously developed calibration curves for loamy soils. Water in the pots with adequate soil moisture was replenished when SWP reached a value of -1.3 bars. In the water stressed pots, water was replenished when SWP reached a value of -6.5 bars. All treatments were randomized within clone genotype.

In June and July 1998, leaf gas exchange rates of all male and female ($n = 72$) clones were measured using a Licor 6400 photosynthesis system. The system we used was equipped with a light source that enabled us to fix photosynthetic active radiation (PAR) at 1500 (± 5) μmol m^{-2}. Gas exchange measurements of CO_2 and water vapour were used to calculate net photosynthesis and transpiration rates (A and E) and stomatal conductance to water vapour (g_{sw}).

In August 1998, we finalized the experiment and harvested shoot biomass from all 108 clones, and root biomass from a subset of 54 clones corresponding to half the genotypes (three of each gender) randomly selected from the total set of 18 genotypes. Shoot biomass was separated into current year's growth and previous year's growth, placed into labelled bags, oven-dried and weighed to the closest mg. Root biomass was determined by emptying the content of each of 54 pots into a 10 mm screen placed on a 40 l plastic container. Larger roots were separated from the soil using a mild pressure water jet, and finer roots (that passed through the 10 mm sieve) were retrieved from the 40 l container through flotation separation with a 2 mm sieve. After the initial washing procedure all root material was re-washed in separate containers and finer roots were once again retrieved through flotation with 2 and 1 mm sieves. Roots were then placed on labelled trays and oven-dried at 60 °C. Remaining soil residue was removed prior to weighing dry root biomass, weights were recorded to the closest mg.

Data were analysed using a mixed effects analysis of variance model. We evaluated the effect of shrub gender, defoliation regime, soil moisture regime, and genotype (nested within gender), on eight productivity-related responses. The response variables considered were above-ground net primary production (ANPP) and shoot biomass (SB), including biomass removed by clipping, for all 108 clones. We analysed root biomass (RB), total shoot and root biomass (TB), and shoot:root ratio (SRR) for the subset of 54 clones mentioned above. Net photosynthesis rate (A), transpiration rate (E) and stomatal conductance to water vapor (g_{sw}) were evaluated in all 72 female and male clones. Shrub gender, defoliation regime and soil moisture regime were considered fixed effects; genotype was considered a random effect. All interactions between main fixed effects were considered in the model. Significance level was set at $p<0.05$. We used the SNK method to compare significantly different means of levels of main factors. Mean comparisons for significant interaction terms were performed using the least square means method controlling for comparisonwise error rates. We used PROC GLM and PROC MIXED from the SAS Version 6.12 (1996) package to perform the statistical analyses.

The overwhelming effect of the soil moisture treatment we imposed obscured the effects of clone gender and defoliation level on the leaf gas exchange variables (A, E, and g_w) analysed individually. Therefore, we re-analysed these data using discriminant analysis to determine whether a suite of predictors composed of the individual values of A, E, and g_w for the months of June and July plus the mean of both measurements, could discriminate significantly among different defoliation groups within clone gender (female or male) for each soil moisture level (adequate soil moisture or water stress). We used Roy's Greatest Root as the statistical test, and again established the significance level at $p<0.05$ (Afifi and Clark, 1996). Finally, to explore the water use efficiency of male and female clones we compared the parameters of the linear regression equations that described the relation between mean A and g_w of female and male clones at each soil moisture level. We used t-tests to compare the slopes of regression functions. We used PROC DISCRIM, PROC GLM and PROC REG of the SAS Institute (1996) version 6.12 package to perform the statistical analyses.

3. Results

3.1. Whole plant responses

Shrub gender had a significant effect on almost all whole-plant response variables we measured (Tables 1 and 2). Clipping treatments significantly affected RB, total shoot and root biomass (TB), and shoot:root ratio (SRR) (Table 2). Clone genotype significantly affected all of the above-ground responses we measured (Table 1). All above- and below-ground whole plant responses were significantly affected by the watering treatments we imposed (Tables 1–3).

3.1.1. Conditions of adequate soil moisture

Whereas ANPP and SB of non-defoliated clones in all gender groups was similar, moderately and heavily defoliated female clones often produced less shoot biomass than defoliated male or monecious clones (Fig. 1a and b). ANPP and SB of control and defoliated monecious clones were not significantly different from that of their

Table 1
Effects of shrub gender, defoliation, soil moisture and genotype on ANPP and total shoot biomass measured on 108 *Atriplex canescens* clones

		ANPP		Total shoot biomass	
	df	F	*p*	F	*p*
Fixed effects					
Shrub gender	2	4.20	0.04	5.55	0.01
Defoliation	2	2.04	0.14	0.65	0.53
Soil moisture	1	28.63	<0.01	24.46	<0.01
Shrub gender*defoliation	4	1.47	0.22	1.96	0.11
Shrub gender*soil moisture	2	0.58	0.56	0.47	0.63
Defoliation*soil moisture	2	0.87	0.42	0.57	0.57
Shrub gender*defoliation*soil moisture	4	0.65	0.69	0.46	0.77
Random effect					
Genotype (Shrub gender)	15	2.03	0.02	2.92	<0.01

Table 2
Effects of shrub gender, defoliation, soil moisture and genotype on root biomass, total (root + shoot) biomass, and shoot: root ratios measured on a subset of 54 *Atriplex canescens* clones

		Root biomass		Total (root + shoot) biomass		Shoot:root ratios	
	df	F	*p*	F	*p*	F	*p*
Fixed effects							
Shrub gender	2	5.90	0.04	5.01	0.05	0.03	0.97
Defoliation	2	8.86	<0.01	10.78	<0.01	3.50	0.04
Soil moisture	1	3.68	0.07	6.69	0.02	13.98	<0.01
Shrub gender* defoliation	4	0.85	0.51	2.7	0.05	0.82	0.52
Shrub gender*soil moisture	2	0.97	0.39	0.80	0.46	0.33	0.72
Defoliation*soil moisture	2	3.33	0.05	6.06	0.01	0.76	0.48
Shrub gender*defoliation*soil moisture	4	1.53	0.22	0.44	0.77	1.56	0.21
Random effect							
Genotype (Shrub gender)	6	0.88	0.52	2.03	0.02	2. 22	0.07

Table 3
Effects of soil moisture treatments on leaf gas exchange response variables of all male and female fourwing saltbush clones ($n = 72$)

Response variable	df	F	*p*
Photosynthesis rate (A)	1	29.14	<0.01
Transpiration rate (E)	1	30.72	<0.01
Stomatal conductance to water vapour (g_w)	1	31.46	<0.01

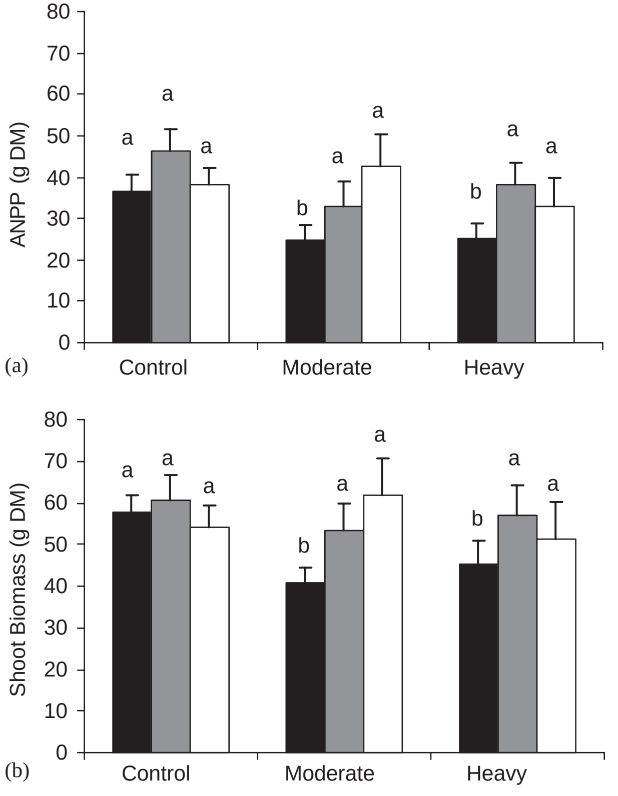

Fig. 1. Above-ground net primary production (a) and shoot biomass (b) of female (full bars), monecious (hatched bars), and male (empty bars) fourwing saltbush clones subjected to three defoliation treatments with adequate levels of soil moisture. Different letters above bars indicate significant differences ($p = 0.05$) between means of clones within each defoliation treatment. Bars indicate SE of the means.

male counterparts. Thus, female clones were the most negatively affected by the defoliation treatments that we imposed. Non-defoliated monecious and female clones produced the most and least RB (root biomass), respectively (Fig. 2a), whereas all defoliated clones had similar RB irrespective of gender group. Total root and shoot biomass mirrored the behaviour of ANPP and SB of the entire set of 108 clones described above (Fig. 2b). Undefoliated females had the highest SRR, and were therefore the gender group that allocated the highest proportion of total plant biomass to the root system. Conversely, moderately defoliated female clones exhibited the lowest SSR. Heavily defoliated clones in all three gender groups exhibited similar SSR (Fig. 2c).

3.1.2. Conditions of soil moisture deficit

Overall, water stress significantly depressed biomass production and leaf gas exchange rates and promoted increases in the relative amount of biomass allocated

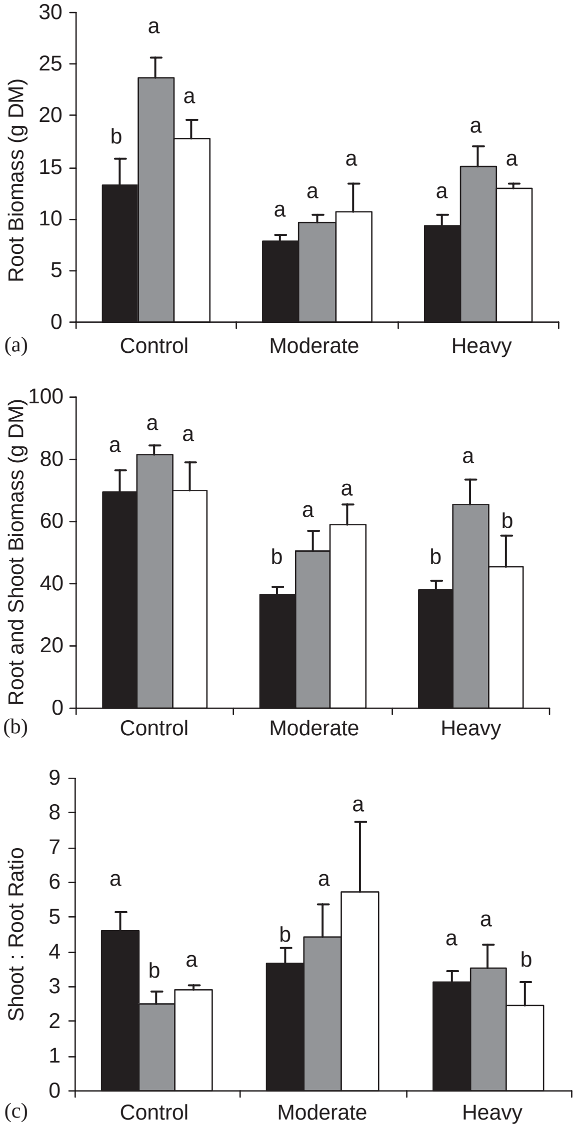

Fig. 2. Root biomass (a), total shoot and root biomass (b) and shoot:root ratios (c) of female (full bars), monecious (hatched bars), and male (empty bars) fourwing saltbush clones subjected to three defoliation treatments with adequate levels of soil moisture. Different letters above bars indicate significant differences ($p = 0.05$) between means of clones within each defoliation treatment. Bars indicate SE of the means.

to root systems irrespective of gender and defoliation treatment. Non-defoliated water-stressed monecious clones exhibited the highest ANPP and SB values (Fig. 3a and b). Defoliated water-stressed clones, however, produced similar amounts of

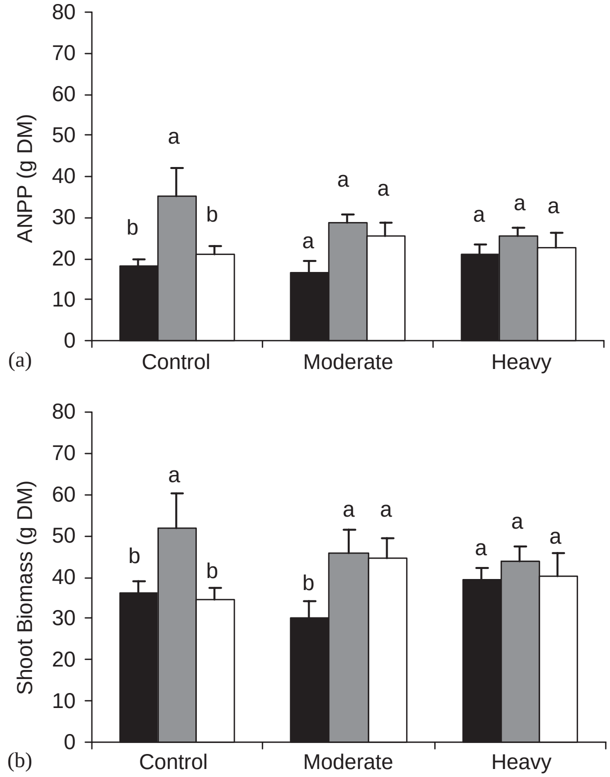

Fig. 3. Above-ground net primary production (a) and shoot biomass (b) of female (full bars), monecious (hatched bars), and male (empty bars) fourwing saltbush clones subjected to three levels of defoliation under conditions of water stress. Different letters above bars indicate significant differences ($p = 0.05$) between means of clones within each defoliation treatment. Bars indicate SE of the means.

biomass irrespective of gender. Moderately defoliated females tended to produce less SB, RB and TB than either male or monecious clones (Figs. 3b, 4a and b), whereas the RB and TB of non-defoliated controls or of heavily defoliated clones was not significantly different among gender groups. SRR of water stressed clones in all three gender groups was similar (Fig. 4c).

3.2. Leaf gas exchange responses

3.2.1. Discriminant analysis results

Female clones in the adequate soil moisture and water stress treatments were classified into significantly different defoliation groups on the basis of leaf gas exchange variables (Table 4). Defoliated female clones tended to exhibit higher photosynthesis and transpiration rates than controls (Table 5). Over 80% of all female clones were correctly classified into either control, moderate or heavy

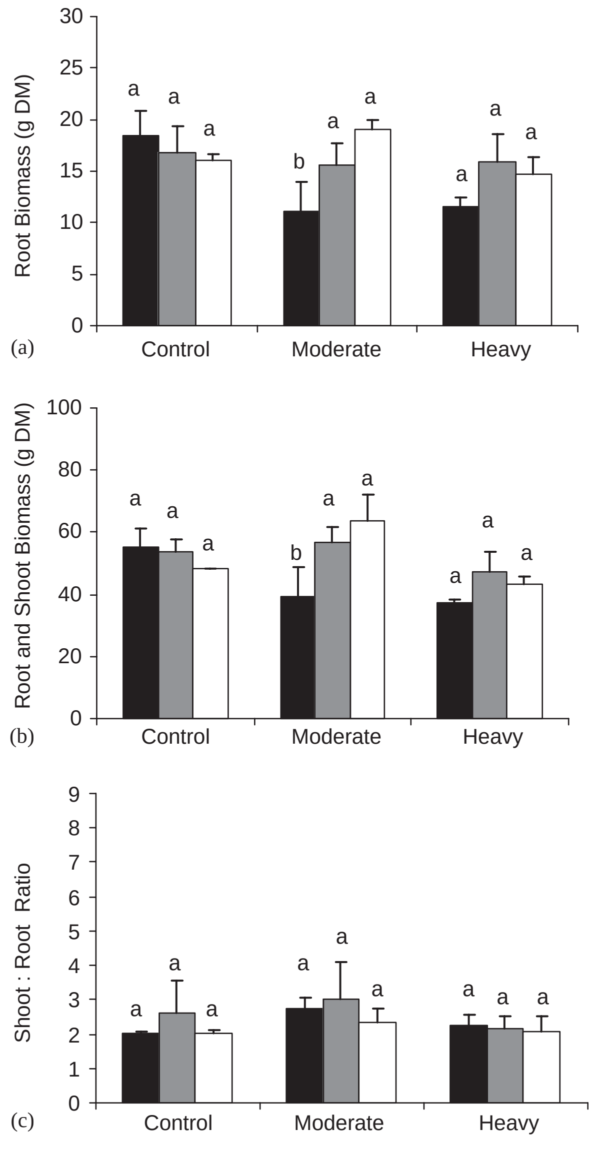

Fig. 4. Root biomass (a), total shoot and root biomass (b) and shoot:root ratios (c) of female (full bars), monecious (hatched bars), and male (empty bars) fourwing saltbush clones subjected to conditions of water stress. Different letters indicate significant differences ($p = 0.05$) between means of clones within each defoliation treatment. Bars indicate SE of the means.

defoliation groups using the leaf gas exchange rate as the classification criterion. Gas exchange rates of male clones, on the other hand, did not allow the discrimination of significantly different defoliation groups (Tables 4 and 5).

Table 4
Discriminant functions used to classify individual leaf gas exchange observations (A: net photosynthesis rate; g_{sw}: stomatal conductance to water vapor; E: transpiration rate) intro groups on the basis of defoliation treatment (c: control; h: heavy defoliation; m: moderate defoliation)

	Coefficients of discriminant functions											
	Females—no water stress			Females—water stress			Males—no water stress			Males—water stress		
Variables	c/h	c/m	h/m	c/h	c/m	h/m	c/h	c/m	h/m	c/h	c/m	h/m
Constant	7.9	1.9	−5.9	15.6	0.8	−14.8	−0.2	−1.3	−1.2	1.9	−2.0	−3.9
A Measured in June	516.8	72.0	−444.8	−1003.1	−242.4	760.8	−767.2	−499.4	267.8	−755.7	−673.9	81.9
g_{sw} Measured in June	1373.0	−1020.0	−2393.0	−1222.9	−7.7	1215.3	412.9	400.9	−12.0	421.0	464.0	43.1
E Measured in June	−3.6	−0.34	3.3	33.2	8.11	−25.1	0.2	−0.3	−0.6	7.3	4.9	−2.4
A Measured in July	514.9	70.0	−444.9	−1004.5	−242.8	761.7	−769.5	−500.5	269.0	−799.2	−673.7	125.4
g_{sw} Measured in July	1847.0	−948.0	−2795.0	1262.8	681.8	−581.0	935.9	605.7	−330.1	555.0	1211.5	656.5
E Measured in July	−25.4	7.5	32.9	−11.7	−7.2	4.5	−10.2	−3.8	6.4	6.4	−17.3	−23.6
Mean A for June and July	−1028.0	−142.0	886.0	2007.2	484.8	−1522.4	1537.4	1000.2	−537.1	1599.8	1345.8	−254.0
Mean g_{sw} for June and July	−2264.0	1775.0	4039.0	−2567.0	−1149.0	1418.0	−1120.4	−878.3	242.1	−1853.3	−1598.0	255.3
Mean E for June and July	−14.27	0.7	15.0	57.8	13.2	−44.6	−1.5	−1.4	0.1	12.4	6.7	−5.6
MANOVA statistics												
F		3.2			5.1			2.4			1.6	
Roy's greatest root		2.2			3.6			1.6			1.1	
p		0.05			0.01			0.1			0.24	

Table 5
Treatment means[a] for photosynthesis rate (A; mg CO^2 m^{-2} s^{-1}), stomatal conductance to water vapour (g_w; cm s^{-1}) and transpiration rate (E; mg H_2O m^{-2} s^{-1}) of fourwing saltbush clones

		No water stress			Water stress		
		A	g_w	E	A	g_w	E
Females	Control	7.6	0.06	2.1	5.1	0.03	0.9
	Moderate	8.5	0.07	2.2	6.5	0.04	1.3
	Heavy	8.4	0.07	2.3	5.3	0.06	1.9
Males	Control	9.7	0.07	2.4	7.2	0.04	1.4
	Moderate	8.3	0.07	2.3	4.7	0.03	1.1
	Heavy	7.9	0.06	2.1	5.2	0.03	0.9

[a]Means of June and July measurements.

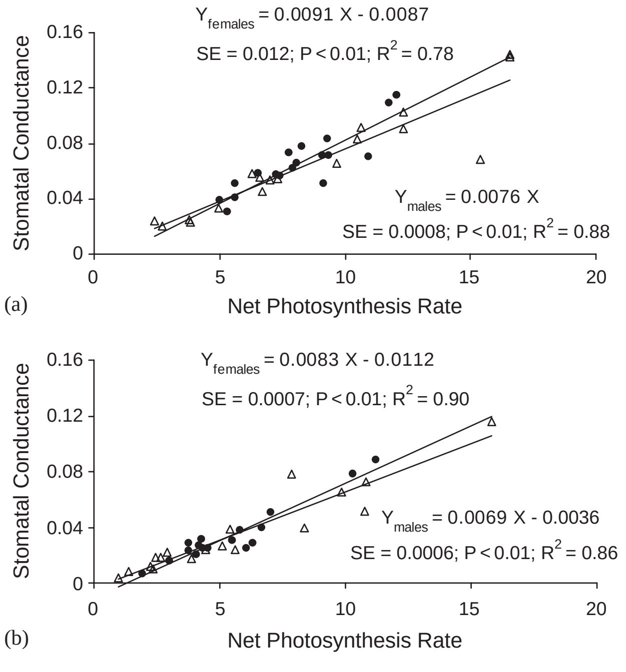

Fig. 5. Linear relation between photosynthesis rate (A; mg CO_2 dm^{-2} h^{-1}) and stomatal conductance to water vapour (g_w; cm s^{-1}) in male (triangles) and female (circles) fourwing saltbush clones subjected to conditions of adequate soil moisture (a) or water stress (b). S.E. values for β_1 of each function are given.

3.2.2. Regression analysis results

Increases in A (photosynthesis rate) were associated with significant increases of g_w (stomatal conductance to water vapour) in all clones irrespective of gender and watering treatment (Fig. 5). The slope of the linear relation between A and g_w in water-stressed female clones was steeper than that of their male counterparts ($\beta_{1\text{Females}} = 0.0083$; $\beta_{1\text{Males}} = 0.0069$; df $= 16$; $t = 2.21$; $p = 0.04$). Therefore, at equal levels of A, females tended to exhibit higher levels in g_w than did males and were therefore probably less efficient in the use of water. Under conditions of adequate soil moisture, we observed a similar trend, but the difference between males and females did not reach statistical significance ($\beta_{1\text{Females}} = 0.0091$; $\beta_{1\text{Males}} = 0.0076$; df $= 16$; $t = 1.92$; $p = 0.07$).

4. Discussion

Female and male individuals of many common rangeland dioecious plant species differ in their use of critical resources for growth such as water and nitrogen (*Atriplex canescens*, Freeman and McArthur, 1982; *Acer negundo*, Dawson and Ehleringer, 1993; *Salix planifolia,* Houle, 1999; *Ilex aquifolium*, Retuerto et al., 2000; *Poa ligularis*, Bertiller et al., 2002). Dioecious plant species also exhibit gender-related differences both in their likelihood of being grazed and in their response to herbivory (*S. lasiolepis;* Boecklen et al., 1990; *Carex picta,* Delph et al., 1993; *Ephedra trifurca,* Boecklen and Hoffman, 1993; *A. canescens*, Maywald et al., 1998; Cibils et al., 2003). The latter, however, is possibly the attribute that has showed least consistent responses across dioecious species. Because gender-specific differences in growth rates are sensitive to environmental conditions (Retuerto et al., 2000), the difficulty to derive a general pattern of gender-specific response to herbivory could be associated with the influence that growing conditions (i.e. availability of moisture or other critical resources) exert on the defoliated plant.

Gender-specific responses to defoliation of fourwing saltbush clones in our experiment appeared to be context-specific both in terms of above- and below-ground biomass. Both intensity of defoliation and soil moisture conditions influenced the performance of each gender relative to its counterparts. Clipped female clones produced the least shoot biomass when soil moisture was adequate, presumably because they recovered more slowly from defoliation than did male and monecious clones. Under conditions of water stress (especially under heavy defoliation), all genders produced similar amounts of shoot biomass. Although gender-specific root biomass responses were also context-related, they exhibited the opposite trend. Under conditions of adequate soil moisture all defoliated clones produced similar amounts of root biomass regardless of clone gender. Defoliated water-stressed females, on the other hand, often produced less root biomass than either their male or monecious counterparts. Therefore, defoliated females were the gender that produced the least amount of shoots when soil moisture levels were adequate, and the least amount of root biomass when soil moisture was deficient.

Physiological differences in the way female and male fourwing saltbush clones responded to defoliation under contrasting levels of soil moisture availability may partly explain the variation in the gender-specific responses observed. Defoliation appeared to stimulate gas exchange rates in leaves of defoliated female clones, particularly under conditions of water stress. Conversely, defoliation of male clones showed a tendency to slow down gas exchange, although the differences we measured did not reach statistical significance. The relations between photosynthesis rates and stomatal conductance to water vapor (A/g_w) suggest that, as observed in other dioecious species (Dawson and Ehleringer, 1993; Retuerto et al., 2000), male fourwing saltbush clones in our study used water more conservatively than did females. Differences in the A/g_w relation suggest that carbon capture was a more costly process (in terms of moisture loss) for females than it was for males. Hence, even though defoliation tended to increase gas exchange rates of females, lower water use efficiency may have caused them to regrow at slower rates than their more efficient male counterparts. Interestingly, male clones tended to exhibit higher levels of total leaf N than did females as was also reported by Tiedemann et al. (1987). Water stressed plants tended to have higher levels of leaf N than clones grown under adequate soil moisture conditions (Cibils et al., unpublished data). Much of the leaf N of water-stressed saltbush clones, however, may have been tied up in non-protein compounds (Wynn Jones and Storey, 1981; Trione and Passera, 1993) that allow them to adjust osmotically and survive under conditions of moisture deficit.

Differences in water use efficiency measured at the level of individual leaves may have influenced the contrasting shoot:root allocation patterns we observed. Such allocation patterns were possibly the proximal causes of the context-specific responses we observed. Under conditions of adequate soil moisture, moderately defoliated male and monecious clones appeared to invest relatively more resources in developing shoots. Female clones, on the other hand, exhibited fairly rigid above:below-ground biomass ratios that may have constrained their ability to compensate for the shoot biomass removed; their relative inefficiency in the use of water may have forced them to invest heavily in maintaining a large root system. Under conditions of soil moisture stress, defoliated male and monecious clones appeared to prioritize investment of resources in root systems, as occurs in other species subjected to resource limitation (Steen, 1984; Houle, 1999). Therefore, above-ground biomass compensation levels for all 3 genders were similar. However, water-stressed defoliated female clones produced significantly less root biomass than male and monecious clones possibly because, once again, they were constrained by their relative inefficient use of water and were unable to produce sufficient photosynthates to sustain an adequate root system.

Monecious clones usually exhibited intermediate responses except in situations where stress was highest. Under conditions of heavy defoliation and adequate soil moisture (photosynthesis impairment) or under conditions of no defoliation and severe soil water deficit (maximum internal water stress) monecious clones outperformed their male and female counterparts. All monecious fourwing saltbush shrubs are sexually labile (McArthur, 1977; McArthur et al., 1992), a characteristic that allows them to shift gender expression in response to environmental stress. This trait may also be associated with the ability to undergo physiological adjustments that enable them to remain productive and survive under conditions of extreme stress (but see Tiedemann et al., 1987). Our results suggest that defoliated female fourwing saltbush shrubs are at a competitive disadvantage regardless of the environmental context, even when they did not flower and produce seed. Their relative inefficiency in the use of water and apparent limited flexibility in above- to below-ground biomass allocation patterns, could cause them to do more poorly than defoliated male or monecious shrubs in both moist and dry soil conditions. Females should, therefore, be the shrub gender most negatively affected by herbivory. Conditions of heavy browsing should promote not only shifts in phenotypic sex ratios as reported by Cibils et al. (2000), but should also alter the proportion of sexually stable: sexually labile individuals, dramatically increasing the frequency of the latter.

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